DP Barcode: D215429 MRID No.: 436200-02

DATA EVALUATION RECORD
§ 72-3(B) -- ACUTE EC_{50} TEST WITH AN ESTUARINE/MARINE MOLLUSK SHELL DEPOSITION STUDY

1. **CHEMICAL:** Didecyl Dimethyl (DDAC) Ammonium Chloride — PC Code No.: 069149

2. **TEST MATERIAL:** 1) DDAC — Purity: 1) 80.5%
   2) [^{14}C]DDAC — 2) 99.4%

3. **CITATION**

Author: Emily Dionne
Title: Didecyldimethylammoniumchloride (DDAC): Evaluation in a Static (Recirculated) Acute Toxicity Test with Eastern Oysters, (*Crassostrea virginica*)
Study Completion Date: March 23, 1994
Laboratory: Springborn Laboratories, Inc., Wareham, MA
Sponsor: Lonza Inc., Fair Lawn, NJ
Laboratory Report ID: 93-12-5079
MRID No.: 436200-02
DP Barcode: D215429

4. **REVIEWED BY:** Christian M. Newman, Wildlife Biologist, KBN Engineering and Applied Sciences, Inc.

Signature: C. McNewman (for) **Date:** 11/3/95

APPROVED BY: Pim Kosalwat, Ph.D., Senior Scientist, KBN Engineering and Applied Sciences, Inc.

Signature: P. Kosalwat **Date:** 11/3/95

5. **APPROVED BY:** ~~Ann Stavola~~ HENRY T. CRAVEN, Head of Section ~~5~~ 4, EEB, EFED

Signature: Henry T. Craven 6/21/96 **Date:**

6. **STUDY PARAMETERS**

Age or Size of Test Organism: 29-49 mm valve length
Definitive Test Duration: 96 hours
Study Method: Static
Type of Concentrations: Mean measured

7. **CONCLUSIONS:** This study was conducted using a static system with the test solution circulated within each test chamber. The test procedures used might have led to a greater than 50% decline in all test concentrations from test initiation to 48 hours. The 96-hour EC_{50} obtained from the test (0.11 ppm ai) is questionable. The test should be repeated using a "once-through flow-through" system.

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Results Synopsis
EC_{50}: 0.11 ppm ai 95% C.I.: 0.10-0.12 ppm ai
NOEL: 0.072 ppm ai Probit Slope: N/A

8. ADEQUACY OF THE STUDY

A. Classification: Invalid

B. Rationale: See Section 15

C. Repairability: No

9. BACKGROUND

10. GUIDELINE DEVIATIONS

1. The test was conducted using a static system with circulated solutions within the test chambers.

11. SUBMISSION PURPOSE:

12. MATERIALS AND METHODS

A. Test Organisms

Guideline Criteria	Reported Information
Species Preferred species are the Pacific oyster (*Crassostrea gigas*) and the Eastern oyster (*Crassostrea virginica*)	*Crassostrea virginica*
Mean valve height 25-50 mm along the long axis	38 (± 4) mm
Supplier	P. Cummins Oyster Co., Pasadena, MD
Are all oysters from same source?	Yes
Are all oysters from the same year class?	Not reported

B. Source/Acclimation

Guideline Criteria	Reported Information
Acclimation Period Minimum 10 days	14 days
Wild caught organisms were quarantined for 7 days?	N/A
Were there signs of disease or injury?	No
If treated for disease, was there no sign of the disease remaining during the 48 hours prior to testing?	N/A
Amount of peripheral shell growth removed prior to testing	3-5 mm
Feeding during the acclimation Must be fed to avoid stress.	Algal diet of *Isochrysis galbana* and *Tetraselmis maculata*
Pretest Mortality <3% mortality 48 hours prior to testing	No mortality in the last seven days prior to test initiation

C. Test System

Guideline Criteria	Reported Information
Source of dilution water Natural unfiltered seawater from an uncontaminated source.	Natural unfiltered seawater from the Cape Cod Canal, Bourne, Massachusetts
Does water support test animals without observable signs of stress?	Yes
Salinity 30-34 ‰ salinity, weekly range <6%	32-34 ‰
Water Temperature 15-30° C, consistent in all test vessels	21-22°C
pH	7.1-7.9

Guideline Criteria	Reported Information
Dissolved Oxygen ≥60% of saturation throughout	Range: 64-93% of saturation during the test
Total Organic Carbon	1.3-3.0 mg/L
Test Aquaria Should be constructed of glass or stainless steel.	Glass rectangular chambers (49.5 x 25.5 x 29 cm) with 18 liters of dilution water or test solution
Type of Dilution System Must provide reproducible supply of toxicant	Static test with the test solution continuously circulated within each test chamber by an impeller pump
Flow rate Consistent flow rate	The flow rate generated by a pump within each chamber was 5.25 L per oyster per hour
Was the loading of organism such that each individual sits on the bottom with water flowing freely around it?	Yes
Photoperiod 16 hours light, 8 hours dark	16 hours light, 8 hours dark
Solvents Not to exceed 0.5 ml/L	N/A

D. Test Design

Guideline Criteria	Reported Information
Range Finding Test If EC_{50} >100 mg/L with 30 oysters, then no definitive test is required.	Seventy percent mortality occurred at 0.25 ppm after 96 hours. The EC_{50} and NOEC were 0.1 and 0.063 ppm, respectively.
Nominal Concentrations of Definitive Test Control & 5 treatment levels; each conc. should be 60% of the next highest conc.; concentrations should be in a geometric series	A control, 0.032, 0.055, 0.09, 0.15, and 0.25 ppm ai.

Guideline Criteria	Reported Information
Number of Test Organisms Minimum 20 individual per test level and in each control	20 oysters
Test organisms randomly or impartially assigned to test vessels?	Yes
Biological observations made every 24 hours?	Yes
Water Parameter Measurements 1. Temperature Measured hourly in at least one chamber 2. DO and pH Measured at beginning of test and every 48 h in the high, medium, and low doses and in the control	1. Temperature measured hourly in the dilution water control. 2. DO and pH were measured daily in each chamber.
Was chemical analysis performed to determine the concentration of the test material at the beginning and end of the test? (Optional)	Yes

13. REPORTED RESULTS

A. General Results

Guideline Criteria	Reported Information
Quality assurance and GLP compliance statements were included in the report?	Yes
Control Mortality Not more than 10% of control organisms may die or show abnormal behavior.	0%
Control Shell Deposition Must be at least 2 mm.	2.2 mm
Recovery of Chemical	Mean recovery ranged from 72 to 89% of nominal
Raw data included?	Yes

Guideline Criteria	Reported Information
Signs of toxicity (if any) were described?	Yes, reduced feeding, fecal, and pseudofecal production, as well as gaped shells were observed in oysters exposed to the two highest test concentrations.

Shell Growth

Concentration (ppm)		Number Per Level	Number Dead	Mean Shell Deposition (mm)	Mean Percent Decrease
Nominal	Mean Measured				
Control	<0.008	20	0	2.2	-
Solvent Control	N/A	-	-	-	-
0.032	0.023	20	0	2.9	0(+33)*
0.055	0.049	20	0	2.0	11
0.090	0.072	20	0	1.9	13
0.15	0.13	20	0	1.2	44
0.25	0.20	20	12	0.0	100

*indicates higher shell growth than the control.

B. Statistical Results

Method: Linear regression (Probit analysis)

96-hr EC_{50}: 0.13 ppm ai 95% C.I.: 0.07-0.2 ppm ai

Probit Slope: 6.27 NOEC: 0.072 ppm ai

14. VERIFICATION OF STATISTICAL RESULTS

Parameter	Result
Statistical Method for EC_{50}	Moving average method
EC_{50} (95% C.I.)	0.11 ppm ai (0.10-0.12)
Probit Slope	N/A
Statistical Method for NOEC	Williams' test
NOEC	0.072 ppm ai

15. REVIEWER'S COMMENTS: This study did not follow the SEP or ASTM procedures for an oyster shell deposition test. The study was conducted using a static method with the test solution continuously circulating within each test chamber. Test concentrations decreased by more than half (>50%) in all test solutions by the time the second measurement was conducted (i.e., at 48 hours) (Table 2, attached). In this test, the test solutions were measured at 0, 48, and 96 hours of exposure. The measured concentrations, at 48 hours and 96 hours were similar, but these values were less than half of the initial concentrations. It is not known when the test concentrations reached the 48-hour levels. It is possible that they might have reached those levels shortly after the initiation of the test. By using the mean values estimated from the three measurements (0, 48, 96 hours), the mean measured values could have been overestimated which would have resulted in an underestimation of the DDAC toxicity. Since the EC_{50} obtained from this test (0.11 ppm ai) is borderline between "very highly toxic" and "highly toxic" classifications, it is important that the test be repeated using a "once-through flow-through" system. This study is classified as **Invalid.**

Table 2. Concentrations of DDAC measured in exposure solutions during the 96-hour flow-through exposure of Eastern oysters (*Crassostrea virginica*).

Nominal Concentration (µg A.I./L)	Measured Concentrations (µg A.I./L)							
	0-Hour		48-Hour		96-Hour		Mean (CV)[a]	Percent of Nominal[b]
	#1	#2	#1	#2	#1	#2		
Control	<8.2	<8.3	<8.3	<8.3	<8.3	<8.3	NA[c]	NA
32	45	43	13	15	10	10	23 (74)	72
55	81	100	30	32	22	23	49 (73)	89
90	120	120	51	51	45	44	72 (52)	80
150	180	200	100	110	89	88	130 (39)	85
250	310	330	150	140	140	150	200 (44)	81
QC[d] #1	26.8 (113)[ef]		37.8 (103)		27.4 (107)[f]			
QC #2	38.1 (104)		109 (99.1)		35.4 (96.7)			
QC #3	200 (104)		182 (99.4)		115 (105)			

[a] Mean measured concentrations are presented with the coefficient of variation in parentheses and were calculated using the actual analytical results rather than the rounded (two significant figures) values presented in this table.
[b] Mean percent of nominal = 81%.
[c] NA = Not applicable.
[d] QC = Quality Control sample.
[e] Value in parentheses represents the percent of the nominal fortified concentration for each QC sample.
[f] Percent recovery for this QC sample fell outside of the standard acceptable range established by this laboratory (i.e., outside ± three standard deviations from the mean recovery established during the method validation/recovery study, Appendix VII).

KOSALWAT DDAC CRASSOSTREA VIRGINICA 11-02-95

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CONC.	NUMBER EXPOSED	NUMBER DEAD	PERCENT DEAD	BINOMIAL PROB. (PERCENT)
.19	100	98	98	0
.11	100	65	65	0
.063	100	15	15	0
.023	100	12	12	0

THE BINOMIAL TEST SHOWS THAT .063 AND .11 CAN BE
USED AS STATISTICALLY SOUND CONSERVATIVE 95 PERCENT
CONFIDENCE LIMITS, BECAUSE THE ACTUAL CONFIDENCE LEVEL
ASSOCIATED WITH THESE LIMITS IS GREATER THAN 95 PERCENT.

AN APPROXIMATE LC50 FOR THIS SET OF DATA IS 9.397274E-02

RESULTS CALCULATED USING THE MOVING AVERAGE METHOD

SPAN	G	LC50	95 PERCENT CONFIDENCE LIMITS	
3	1.824793E-02	8.645864E-02	7.840958E-02	9.529951E-02

RESULTS CALCULATED USING THE PROBIT METHOD

ITERATIONS	G	H	GOODNESS OF FIT PROBABILITY
5	3.343794	23.36324	0

A PROBABILITY OF 0 MEANS THAT IT IS LESS THAN 0.001.

SINCE THE PROBABILITY IS LESS THAN 0.05, RESULTS CALCULATED
USING THE PROBIT METHOD PROBABLY SHOULD NOT BE USED.

SLOPE = 3.240735
95 PERCENT CONFIDENCE LIMITS =-2.685288 AND 9.166758

LC50 = 8.116193E-02
95 PERCENT CONFIDENCE LIMITS = 0 AND +INFINITY

LC10 = 3.292047E-02
95 PERCENT CONFIDENCE LIMITS = 0 AND .1076703

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DP Barcode: D215429 MRID No. 432600-03

DDAC(C14)/OYSTER SHELL DEPOSITION TEST
File: h:\project\epa\ddacc14.tox Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	3.055	3.055		
2	0.023	2.700	2.700	0.935	
3	0.037	3.175	3.175	-0.316	
4	0.063	2.590	2.590	1.225	
5	0.11	1.055	1.055	5.268	*

Dunnett table value = 2.21 (1 Tailed Value, P=0.05, df=60,4)

DDAC(C14)/OYSTER SHELL DEPOSITION TEST
File: h:\project\epa\ddacc14.tox Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	20			
2	0.023	20	0.839	27.5	0.355
3	0.037	20	0.839	27.5	-0.120
4	0.063	20	0.839	27.5	0.465
5	0.11	20	0.839	27.5	2.000

DP Barcode: D215429 MRID No. 432600-03

DDAC(C14)/OYSTER SHELL DEPOSITION TEST
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BONFERRONI t-TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	CONTROL	3.055	3.055		
2	0.023	2.700	2.700	0.935	
3	0.037	3.175	3.175	-0.316	
4	0.063	2.590	2.590	1.225	
5	0.11	1.055	1.055	5.268	*

Bonferroni t table value = 2.28 (1 Tailed Value, P=0.05, df=90,4)

DDAC(C14)/OYSTER SHELL DEPOSITION TEST
File: h:\project\epa\ddacc14.tox Transform: NO TRANSFORMATION

BONFERRONI t-TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	CONTROL	20			
2	0.023	20	0.865	28.3	0.355
3	0.037	20	0.865	28.3	-0.120
4	0.063	20	0.865	28.3	0.465
5	0.11	20	0.865	28.3	2.000